

PRODUCT SPECIFICATION SHEET



Esculin Agar (DM302)

Intended Use

Esculin Agar (DM302) is recommended for cultivation and differentiation of bacteria on the basis of esculin hydrolysis and H₂S production.

Product Summary and Explanation

Esculin is a carbohydrate linked to an alcohol that naturally occurs in the horse chestnut (*Aesculus hippocastanum*), California Buckeye (*Aesculus californica*), Prickly Box (*Bursaria spinosa*) and indaphnin (the dark green resin of *Daphne mezereum*). It is also found in dandelion coffee. This glycoside is incorporated as a differential agent to facilitate the identification of various organisms, including *Enterobacteriaceae*, Enterococci and anaerobes. Esculin hydrolysis is recommended in the differentiation and identification of a variety of organisms based on their ability to hydrolyze esculin and produce H₂S.⁽¹⁾ The unhydrolyzed esculin remain unchanged and fluorescence under UV light and can be detected using long wave UV light at 360 nm, whereas hydrolyzed esculin will not fluoresce and medium turns black.^(2, 3)

Principles of the Procedure

Esculin Agar contains casein enzymic hydrolysate and beef heart infusion (solids) provides amino acids and other nitrogenous substances that support bacterial growth. Sodium chloride maintains the osmotic equilibrium of the medium. Esculin is a differentiating agent, which helps in identification of esculin-positive organism. Hydrolysis of esculin yields esculletin and dextrose. In the presence of an iron salt, esculletin forms a brown-black complex that diffuses into the surrounding medium.⁽²⁾

Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	13.00
Sodium chloride	5.00
Yeast extract x	5.00
Beef heart infusion (solids)	2.00
Esculin	1.00
Ferric citrate	0.50
Agar	15.00
Final pH: 7.3 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 41.5 grams of the medium in one liter of distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Distribute into screw capped tubes in 3 ml volumes or as desired.
4. Autoclave at 15 lbs pressure (121°C) for 15 minutes. Cool tubes in a slanted position.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Amber coloured, clear to slightly opalescent gel forms in tubes as slants
Reaction of 4.15% Solution	pH : 7.3 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

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Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Esculin Hydrolysis
1.	<i>Escherichia coli</i> ATCC 25922	50 -100	good	negative reaction
2.	<i>Enterococcus faecalis</i> ATCC 29212	50 -100	good-luxuriant	positive reaction, blackening of medium
3.	<i>Streptococcus pyogenes</i> ATCC 19615	50 -100	good-luxuriant	negative reaction

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Organisms to be tested must first be isolated in pure culture on an appropriate solid medium. Using a sterile inoculating loop or needle, inoculate esculin agar with several isolated colonies.
2. Incubate tubes at 35°C with caps loosened for up to 48 hours. Refer to appropriate references for standard test procedures.

Results

Blackening of the agar medium in the area of growth indicates esculin hydrolysis. Refer to appropriate references and test procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Esculin Agar

Product Code : DM302

Available Pack sizes : 500gm

References

1. Atlas R. M., 1996, Handbook of Microbiological Media, 2nd Ed., CRC Press,
2. Koneman E. W., Allen S. D., Janda W. M., Schreckenberger P. C., Winn W. C. Jr., 1997, Colour Atlas and Textbook of Diagnostic Microbiology, 5th Ed., J. B. Lippincott- Raven Publishers, Philadelphia, Pa.
3. Shigei, 1992, In Isenberg (Ed.), Clinical Microbiology Procedures Handbook, Vol.1, A. Society for Microbiology, Washington, D.C.



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Further Information

For further information please contact your local MICROMASTER Representative.



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